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SEPARATION OF SOME PINK SALMON (Oncorhynchus gorbuscha Walbaum) SUB-POPULATIONS IN PRINCE WILLIAM SOUND, ALASKA BY LENGTH-WEIGHT RELATIONSHIPS AND HORIZONTAL STARCH GEL ELECTROPHORESIS

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ABSTRACT

The generation of a comprehensive salmon hatchery program (public and private) in Alaska necessitates implementation of certain controls to maintain genetic integrity of local indigenous stocks. Controls achieved by identification and classification of potential hatchery donor stock, environmental matching through categorization of stream-types, and coordination with an appropriate genetic policy are deemed essential. Prince William Sound pink salmon stocks (Oncorhynchus gorbuscha Walbaum) (within and among systems) were separated to some extent by length-weight criteria, however, greater refinement of separations was accomplished with starch gel electrophoresis. Thirty-seven subpopulations of even-year pink salmon were reduced, by Roger's coefficient of genetic similarity, to seven stock types. Several protein variants were observed in this study which had never previously been observed in pink salmon.

INTRODUCTION

In 1974 the State of Alaska signed into law an act authorizing the operation of private, non-profit hatcheries. These hatcheries are to be operated without adversely affecting natural stocks of fish in the State and under a policy of management which allows reasonable segregation of returning hatchery-reared salmon from naturally occurring stocks. Alaska State Law, Statute 16.10.445 (1974) states that the Alaska Department of Fish and Game (ADF&G) shall approve the source and number of salmon eggs to be taken from selected donor brood stock and, where feasible, salmon eggs utilized by a hatchery operator shall first be taken from stocks native to the area in which the hatchery is located and then, upon approval by the ADF&G, from other areas as necessary. The term "stock" in this paper shall refer to a group of genetically closely related individuals in a species hence, a variety (Gray 1967).

At this writing, the State of Alaska and private non-profit groups are deeply committed to the construction, operation, and maintenance of hatcheries. The ultimate objective is to reverse a long-term trend of declining salmon production in Alaska (Hunt 1976). State of Alaska policy is that hatcheries will be constructed on streams having depressed or no salmon runs, hence, selection of donor brood stock having similar or matching genetic characteristics (according to the present state of the art) is of considerable importance in maintaining integrity of various local demes.

In regard to locating hatcheries on systems having no salmon runs (due to mechanical or velocity barriers), I assumed, with reference to work done by Wisby and Hasler (1954), that salmon imprint on certain, but undetermined water chemistry attributes of the natal stream. I found that classification of streams by chemical attributes is possible using discriminatory analysis (project in progress). Therefore, I hypothesized that the 871 recognized streams in Prince William Sound could be classified into perhaps 20 stream-types which, in turn, may generally reflect pink salmon genetic variation. Stream classification, however, is being conducted under a separate project and the above stated hypothesis cannot be addressed at this time.

Pink salmon stocks in Prince William Sound exhibit striking differences in spawning distribution between even- and odd-year populations. As described by Noerenberg (1963), during even-years (1972, 1974, 1976, etc.) 72 to 77% of the pink salmon spawn in the intertidal reaches of streams and about 25% spawn farther upstream in the lower freshwater reaches. By contrast, 35 to 57% of odd-year populations spawn intertidally and the remainder spawn in the far upstream reaches. Within even- and odd-year populations, there are further breakdowns by time of run, e.g., early, middle, and late. Thus, for any stream, strength and timing of particular runs are dependent upon in-stream environmental factors which effect survival and development from egg to pre-emergent fry and subsequent ocean survival. The 2 year life cycle of pink salmon prevents even- and odd-year stocks from interbreeding (extremely remote probability/occurrence of 3-year old pink salmon, Scott and Crossman 1973). Thus, even- and odd-year stocks constitute virtually separate genetic lines.

This research was initiated in July 1976 and was designed to identify potential pink salmon donor stocks for present and future hatcheries within Prince William Sound. Only early and late run pink salmon were collected. The middle run was omitted to prevent possible sampling error due to varying stream-life (Helle 1970). Stocks were separated using length and/or weight relations (Mottley 1941; Helle 1970) and enzyme analyses by horizontal starch gel electrophoresis (Utter et al. 1974; May and Utter 1974; Seeb et al. 1975; Milner and Utter 1976).

MATERIALS AND METHODS

Initially, 51 bright, ocean migrant, male and female pink salmon (stock(s) unknown) from a commercial catch in Shelter Bay, Prince William Sound were measured and weighed (Figure 1). Snout to fork (SF) and mid-eye to fork (MEF) lengths were measured with a caliper to the nearest millimeter and regressions established. Weight was measured with a spring scale to the nearest 25 grams. Length-weight relationships were established. Length of both sexes were compared with spawning stocks in pre-selected streams (Figure 1). Weights of females were used as a standard of comparison to possibly detect change in weight of spawning stocks in the pre-selected streams.

Subsequently, MEF and mid-eye to posterior end of hypural plate (MEHP) were measured from known stocks (27 males, 38 females) and conversion from latter to former was calculated. This relationship was established because the caudal fin of females, primarily, is usually worn to a stub during redd digging activities.

Sixteen target streams were selected about Prince William Sound. Stream selection was based on past escapement records and relative distance from each other. Spawning pink salmon were captured in these streams with beach seine and dip nets. Fish were separated into intertidal and freshwater spawners, males and females, and early run and late run. Upon capture the salmon were killed by striking the snout with a club. MEF lengths were taken except when the caudal fin was worn to a stub, whereon MEHP measure-

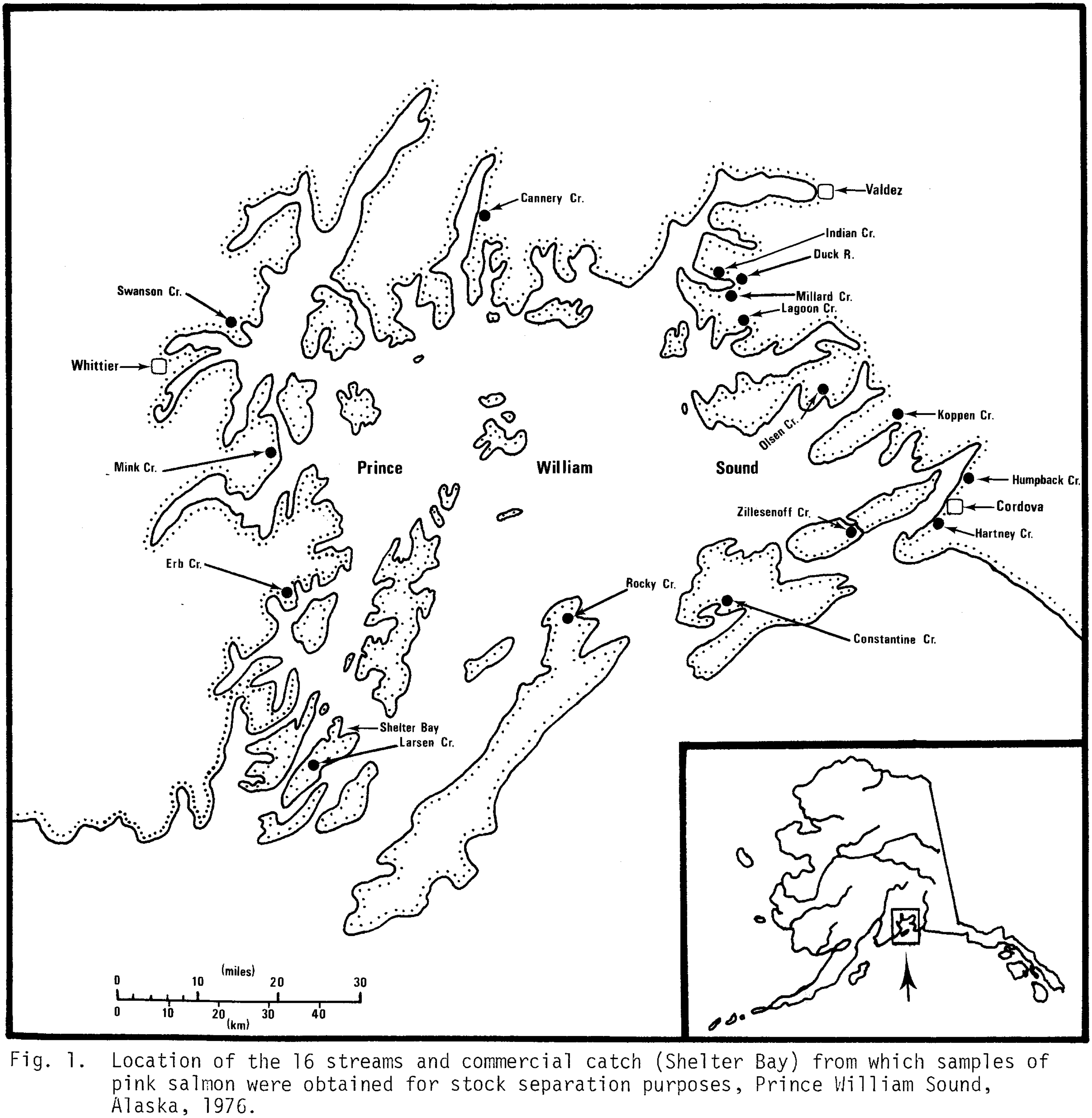

Fig. 1. Location of the 16 streams and commercial catch (Shelter Bay) from which samples of pink salmon were obtained for stock separation purposes, Prince William Sound, Alaska, 1976.

ments were recorded and later converted to MEF. Weight of each specimen was recorded. Data were analyzed by standard statistical methods.

Killed salmon were placed on a portable table (on-site) whereon muscle (a 2 cm x 2 cm x 6 cm block from between the caudal fin and lateral line), liver (minus the gall bladder), and eyes were removed with a sharp, small bladed knife and curve-tip forceps. Tissues from individual fish were placed in small plastic bags with appropriate labels, then placed immediately into an insulated cooler refrigerated with "Blue ice". All individual tissue bags from a designated stream section were placed together in a large plastic bag, also appropriately labeled. The objective was to obtain a minimum sample (randomly selected) of 40 fish per stream section (i.e., 40 intertidal, 40 freshwater). After collection of salmon length-weight data and tissue samples from a stream, the tissues were transported by skiff to the awaiting ADF&G research vessel MONTAGUE, where they were placed in a freezer which held the tissues at -18 °C. After sampling the circuit of 16 streams, the tissues were transferred from the R/V MONTAGUE to a plasma freezer (-35 °C) at the ADF&G laboratory in Cordova. When all samples had been collected (from 1,507 salmon representing 37 sub-populations), they were placed in small insulated "Wet-Lok" boxes packed with dry ice and shipped via commercial airline to Pacific Fisheries Research, Seattle for electrophoretic analysis.

Starch gel electrophoresis, following the method described by May (1975), was used to separate the variant enzyme types. Following electrophoresis, the starch gels were stained for specific enzymes and scored for the frequency of variants.

Initially the fish tissues were screened for variation at approximately 35 genetic loci coding for 35 specific enzymes (Table 1). However, certain enzymes, especially those from the liver, are highly unstable and lose activity within a short period of time. Since a period of 3-4 months elapsed between collection and analysis, the resolution on some of the more unstable enzymes was too poor to be of use. As a result, this report is based on data from 20 loci, 10 of which were polymorphic (Table 2).

Allele frequencies and their 95% confidence intervals were calculated for each of the 10 polymorphic loci in each of the sub-populations. To facilitate analysis of this large quantity of information, a similarity index between each sub-population and every other sub-population was computed. Rogers' Average Similarity Index (Rogers 1972) was chosen for its relative ease of calculation and widespread use. The coefficient of genetic similarity is defined as

$$S = 1 - \frac{1}{L} \sum_{i=1}^{L} \left\{ \frac{1}{2} \sum_{j=1}^{A_i} \left(P_{ijx} - P_{ijy} \right)^2 \right\}^{\frac{1}{2}}$$

Table 1. A list of the enzymes, abbreviation, and tissues initially screened for variation.

Enzyme	Abbreviation[1/]	Tissue
Alcohol dehydrogenase	ADH	Liver
Aspartate aminotransferase	AAT 1, 2 AAT 3	muscle eye
Creatine kinase	CK 1, 2	muscle
Esterase	EST	Liver
Alpha-glycerophosphate	AGP	muscle
Glucose-6-phosphate dehydrogenase	G6P	liver
Isocitrate dehydrogenase	IDH	liver
Lactate dehydrogenase	LDH 1 - 4 LDH 5	muscle eye
Malate dehydrogenase	MDH 1, 2 MDH 3, 4	liver muscle
Malic enzyme	ME	muscle
6-phosphogluconate dehydrogenase	6PG	liver
Phosphoglucomutase	PGM	muscle
Phosphoglucose isomerase	PGI 1 - 3	muscle
Phosphomannose isomerase	PMI	eye
Sorbitol dehydrogenase	SDH 1, 2	liver
Tetrazolium oxidase	TO	liver
Beta glucuronidase dehydrogenase	BGD	muscle
Diaphorase	DIA	liver
Glutathion reductase	GR	liver

1/ Since different genes may code for the same product, each is given a numerical identifier which follows the abbreviation, i.e., LDH 5 is the fifth locus coding for lactate dehydrogenase.

Table 2. A list of 10 polymorphic loci on which the electrophoretic data contained within this study are based.

Enzyme
1. Alpha-glycerophosphate dehydrogenase
2. Phosphoglucomutase
3. Malate dehydrogenase A
4. Malate dehydrogenase B
5. Aspartate aminotransferase 3
6. Lactate dehydrogenase 4
7. Phosphoglucose isomerase 1
8. Phosphoglucose isomerase 3
9. 6-phosphogluconate dehydrogenase
10. Malic enzyme

where L is the number of loci, A_i is the number of alleles at the ith locus, and P_{ijx} and P_{ijy} are the frequency of the jth allele at the ith locus in population x and y, respectively. This index ranges from a value of 1 for identical populations to 0 for those with no shared alleles. These calculated indices were used in conjunction with standard numerical taxonomic techniques (Sneath and Sokal 1973) to generate a dendrogram, or family tree, relating sub-populations by the unweighted average linkage method (UALM). This method was used by Allendorf (1975) to clarify the ambiguous relationships of 38 steelhead stocks in Washington State. A full discussion of these clustering techniques can be found in Sneath and Sokal (1973).

RESULTS

A comparison of MEF and SF lengths for males and females, revealed a significant difference between slopes of the two regressions (Steel and Torrie 1960): $F = 5.6$ (1, 98); $P<0.02$ (Figure 2) which indicated that the distance between the snout to mid-eye was greater in males than females when MEF lengths were the same. To convert female SF length (mm) to MEF length (mm) use regression $y = a_o + a_1x$ or $\hat{y} = 5.5892 + 0.9311\ x$; $r = 0.9931$; $S_{y.x} = 2.3809$; $S_o = 8.1389$; $S_1 = 0.0157$; where y = MEF; x = SF; $S_{y.x}$ = the standard error of estimate of y on x; S_o = the standard error of the regression coefficient a_o; and S_1 = the standard error of the regression coefficient a_1. Conversion of male SF length (mm) to MEF length (mm) is performed using regression $\hat{y} = 25.6321 + 0.8785\ x$; $r = 0.9931$; $S_{y.x} = 2.9667$; $S_o = 7.6395$; $S_1 = 0.0148$.

Figure 3 depicts the regression line and confidence band (weight, g. on MEF length, mm) for commercially caught female pink salmon used as a standard of comparison. Converting male and female MEHP to MEF lengths (mm) implied no difference between slopes of the two regressions: $F = 0.0008$ (1, 61); $P>0.99$. To convert male MEHP (mm) to MEF (mm) use $\hat{y} = -0.4692 + 1.0741\ x$; $r = 0.9941$; $S_{y.x} = 3.2402$; $S_o = 10.3629$; $S_1 = 0.0234$. Conversion of female MEHP (mm) to MEF (mm) is performed using $\hat{y} = -1.7044 + 1.0762\ x$; $r = 0.9932$; $S_{y.x} = 2.5973$; $S_o = 9.7148$; $S_1 = 0.0211$. The combined regression (male and female) to convert MEHP is: $\hat{y} = -0.0029 + 1.0727x$; $r = 0.9945$; $S_{y.x} = 2.8538$; $S_o = 6.5161$; $S_1 = 0.0144$ (Figure 4).

Figures 5 and 6 present MEF length (mm) by sex for all samples collected and Figures 7 and 8 depict weights (g) for most of the samples. Black bars in the figures indicate the 95% confidence interval for the mean. Statistical significance is indicated by the amount of vertical overlap. Non-overlapping black bars of similar size indicate near certainty that samples are significantly different. The white bar at either side of the mean is one standard deviation. As shown in these figures, this standard

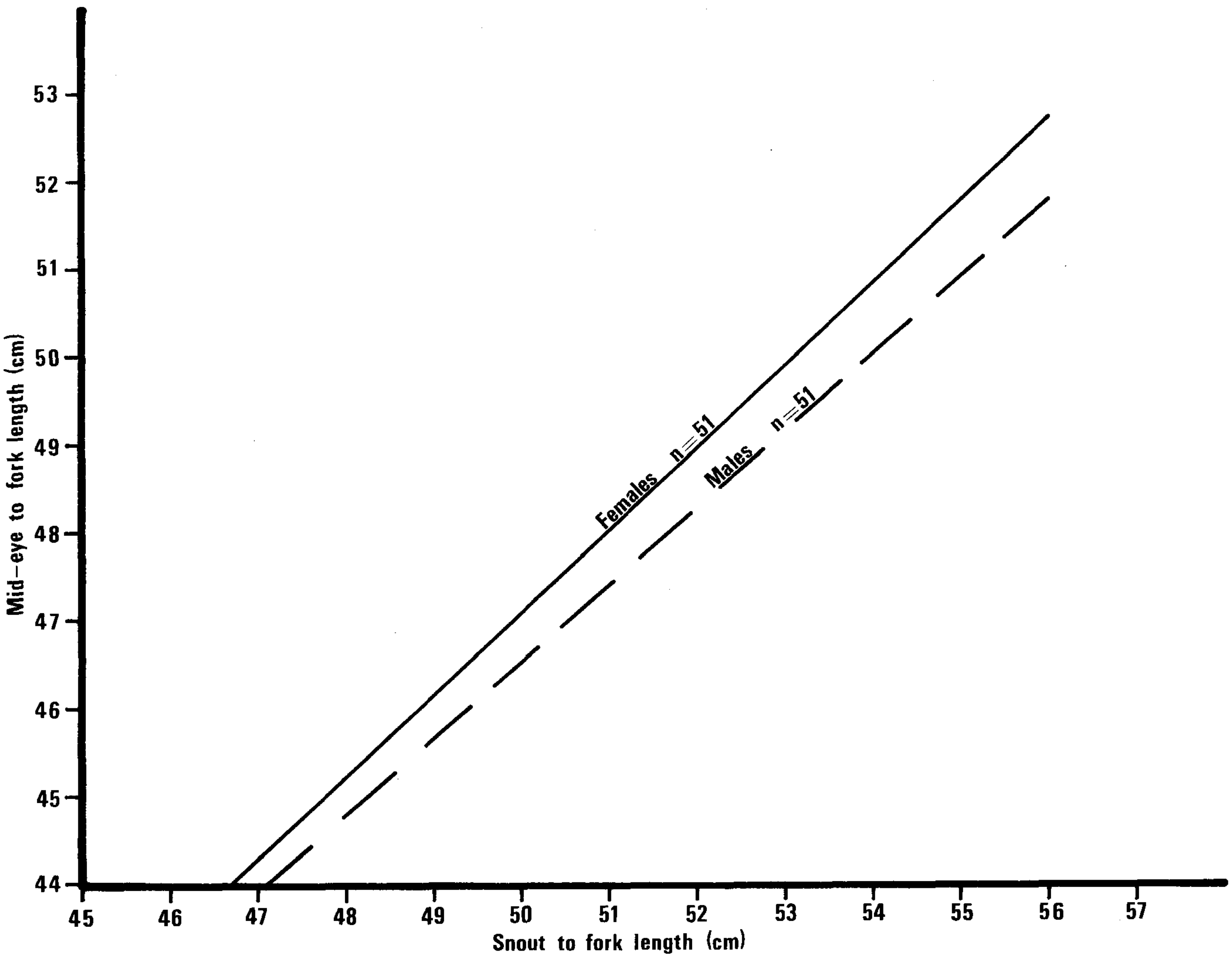

Fig. 2. Regression lines comparing mid-eye to fork length and snout to fork length of pink salmon commercially caught in Shelter Bay, Prince William Sound, 1976.

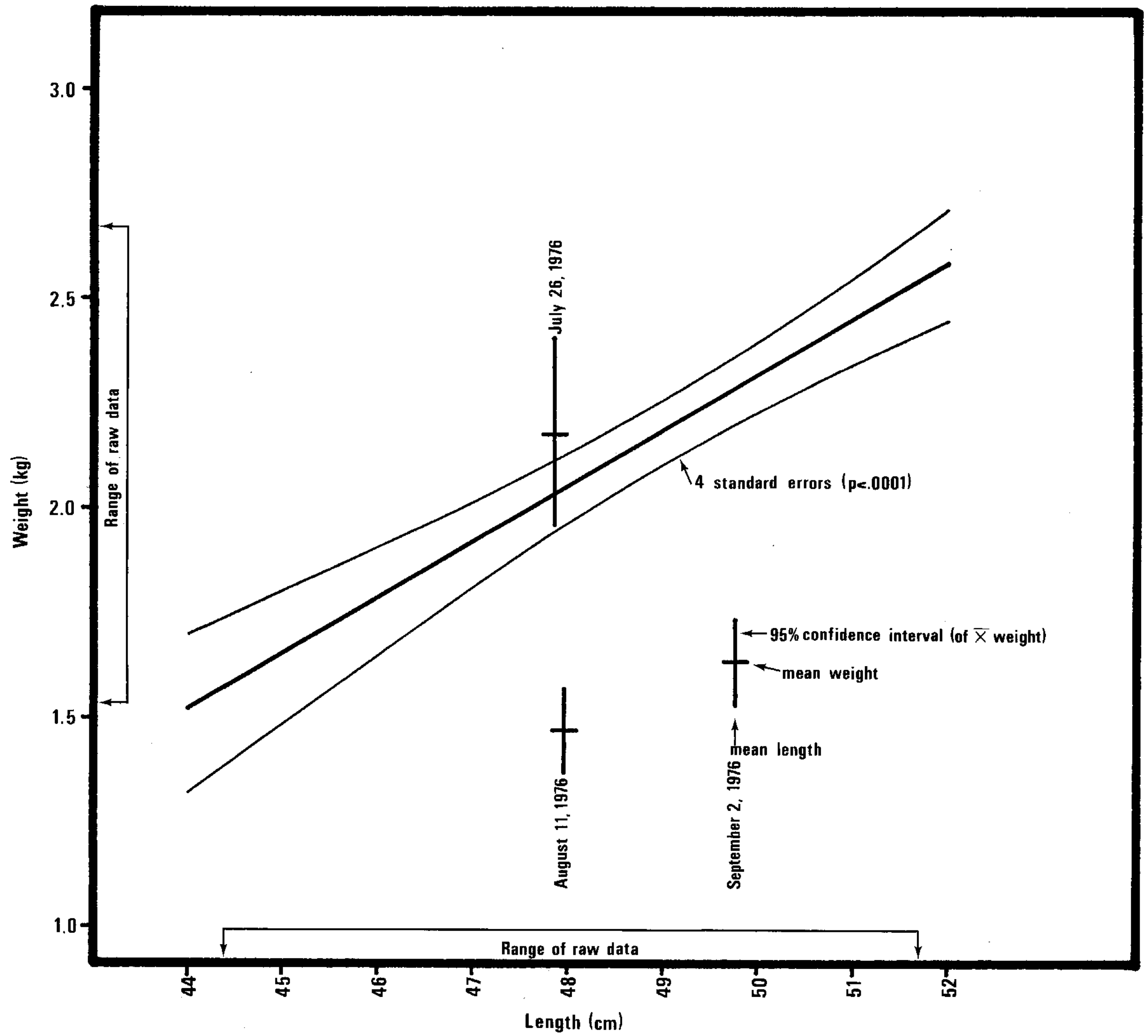

Fig. 3. Regression line and confidence interval for female pink salmon commercially caught in Shelter Bay, Prince William Sound July 23, 1976. This sample of fish was used as a standard of comparison (in this example, against female pink salmon obtained from Humpback Creek July 26, August 11, and September 2, 1976).

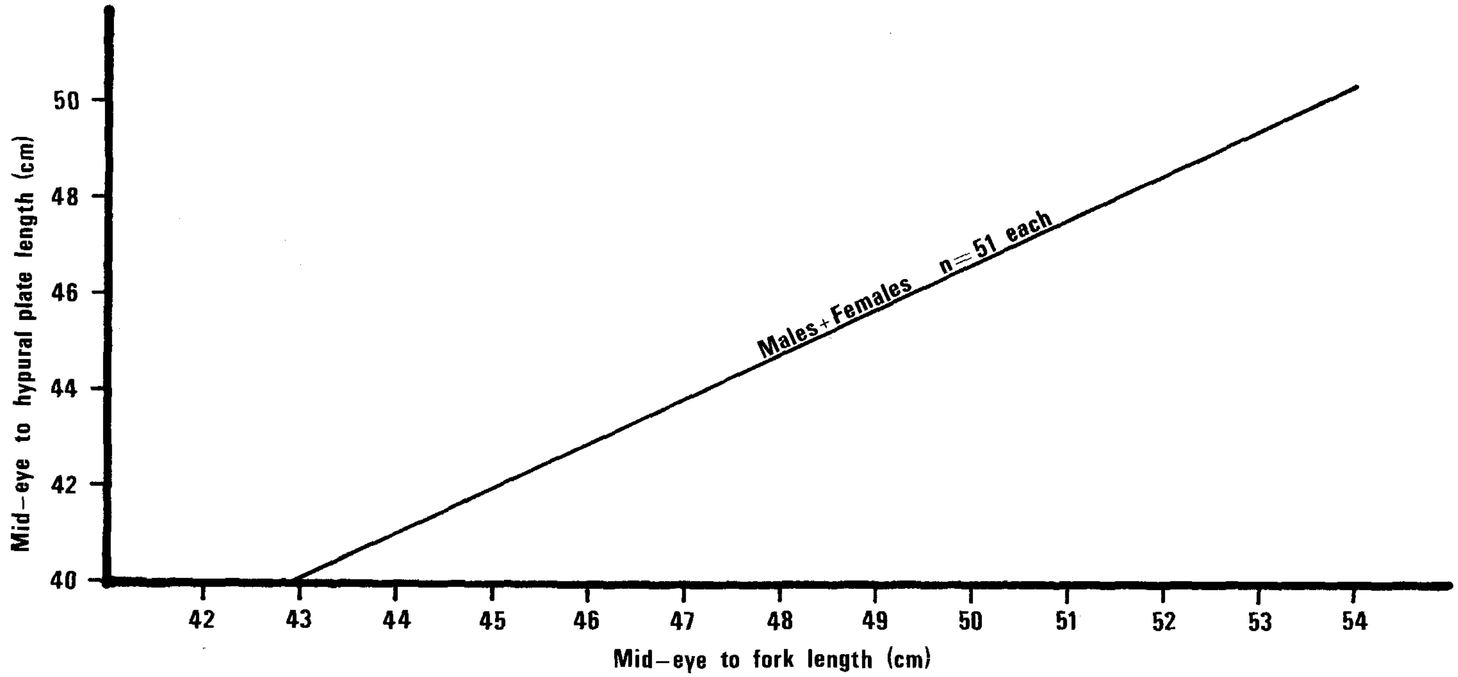

Fig. 4. Regression line converting mid-eye/hypural plate length to mid-eye/fork length of male and female pink salmon commercially caught at Shelter Bay, Prince William Sound, 1976.

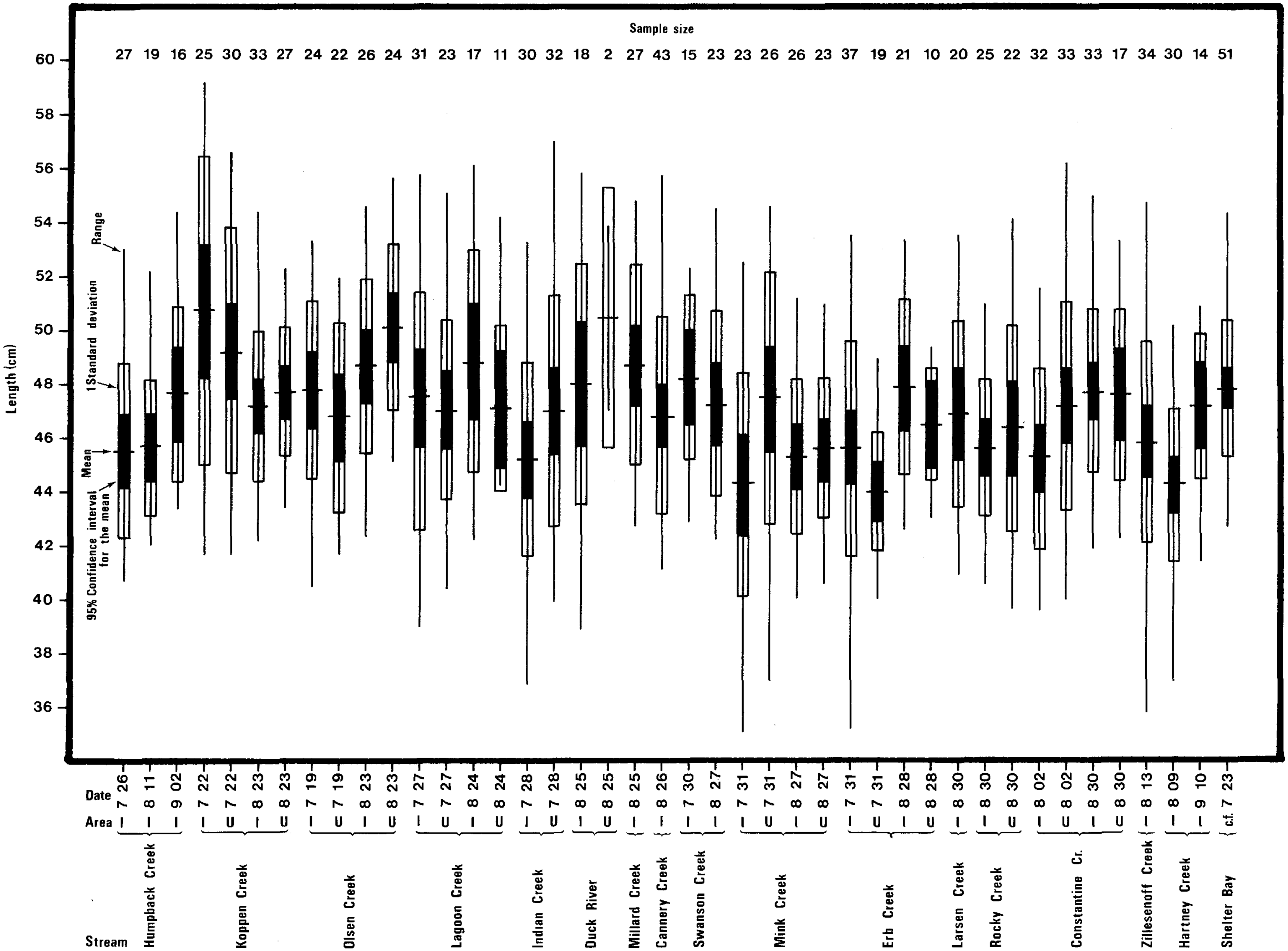

Fig. 5. Mid-eye to fork length of Prince William Sound male pink salmon compared by stream, time and area (I = intertidal, U = upstream, c.f. = commercial fishery), 1976.

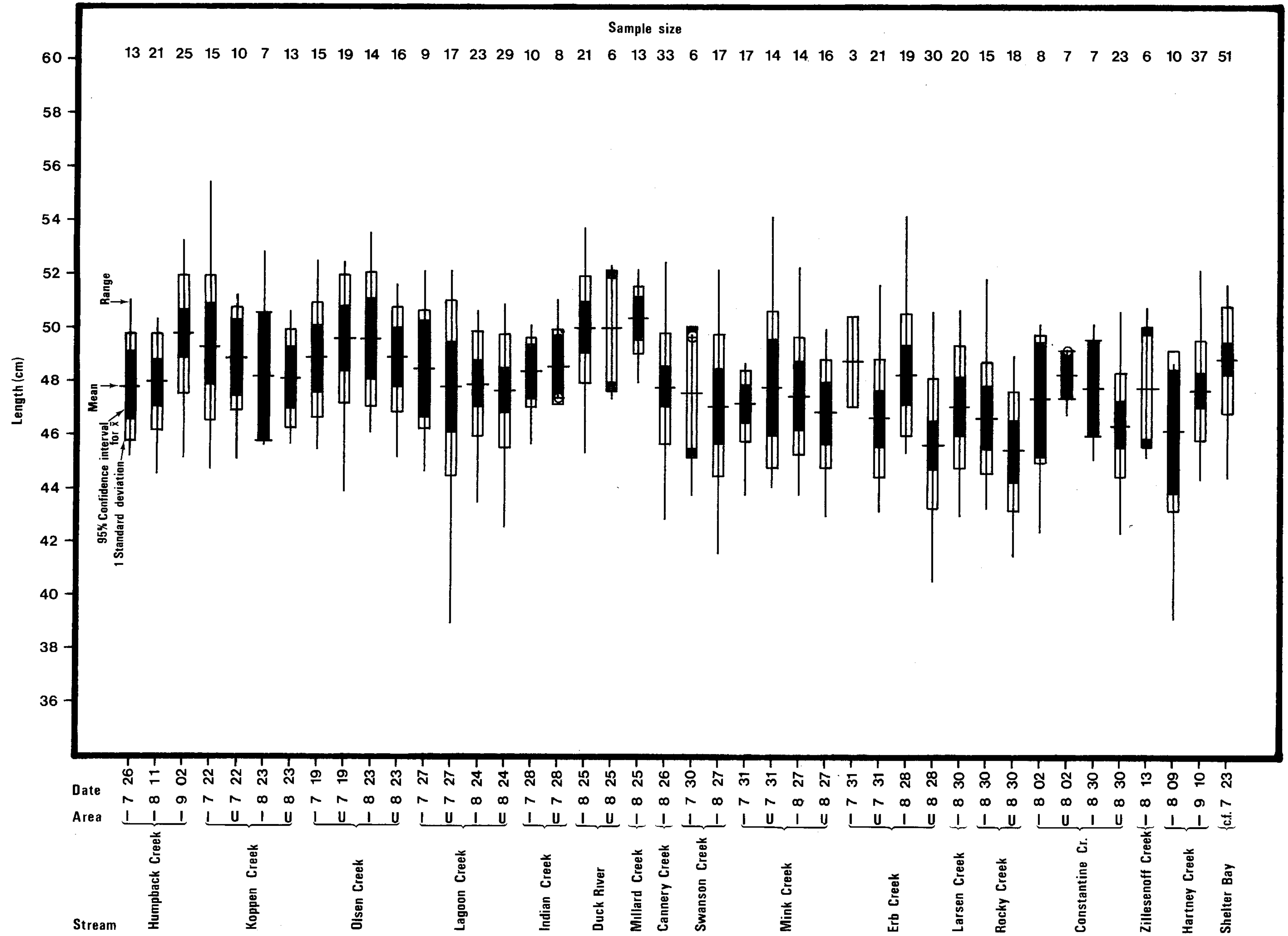

Fig. 6. Mid-eye to fork length of Prince William Sound female pink salmon compared by stream, time and area (I = intertidal, U = upstream, c.f. = commercial fishery), 1976.

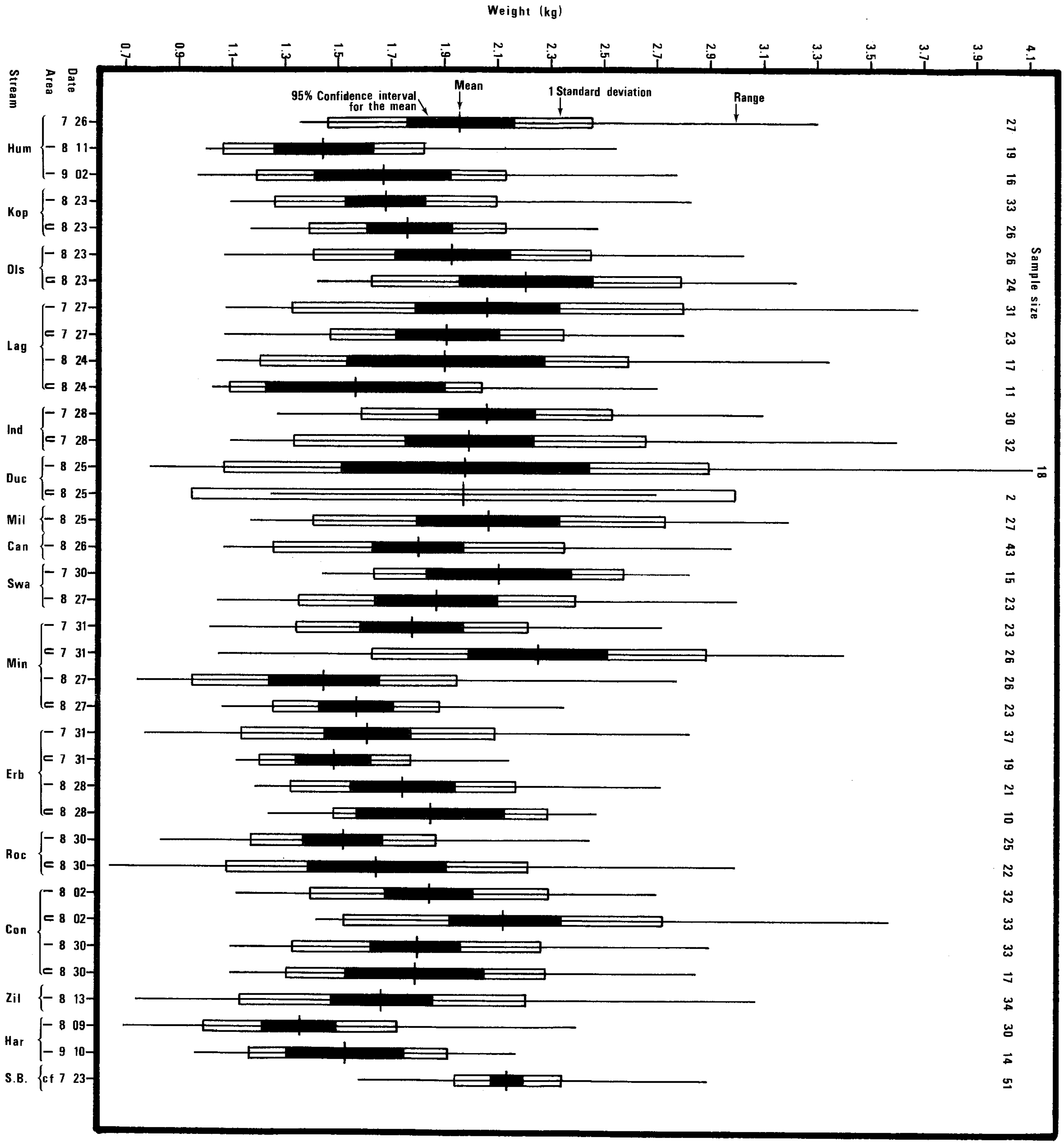

Fig. 7. Total live weight of Prince William Sound male pink salmon compared by stream, time and area (I = intertidal, U = upstream, c.f. = commercial fishery), 1976.

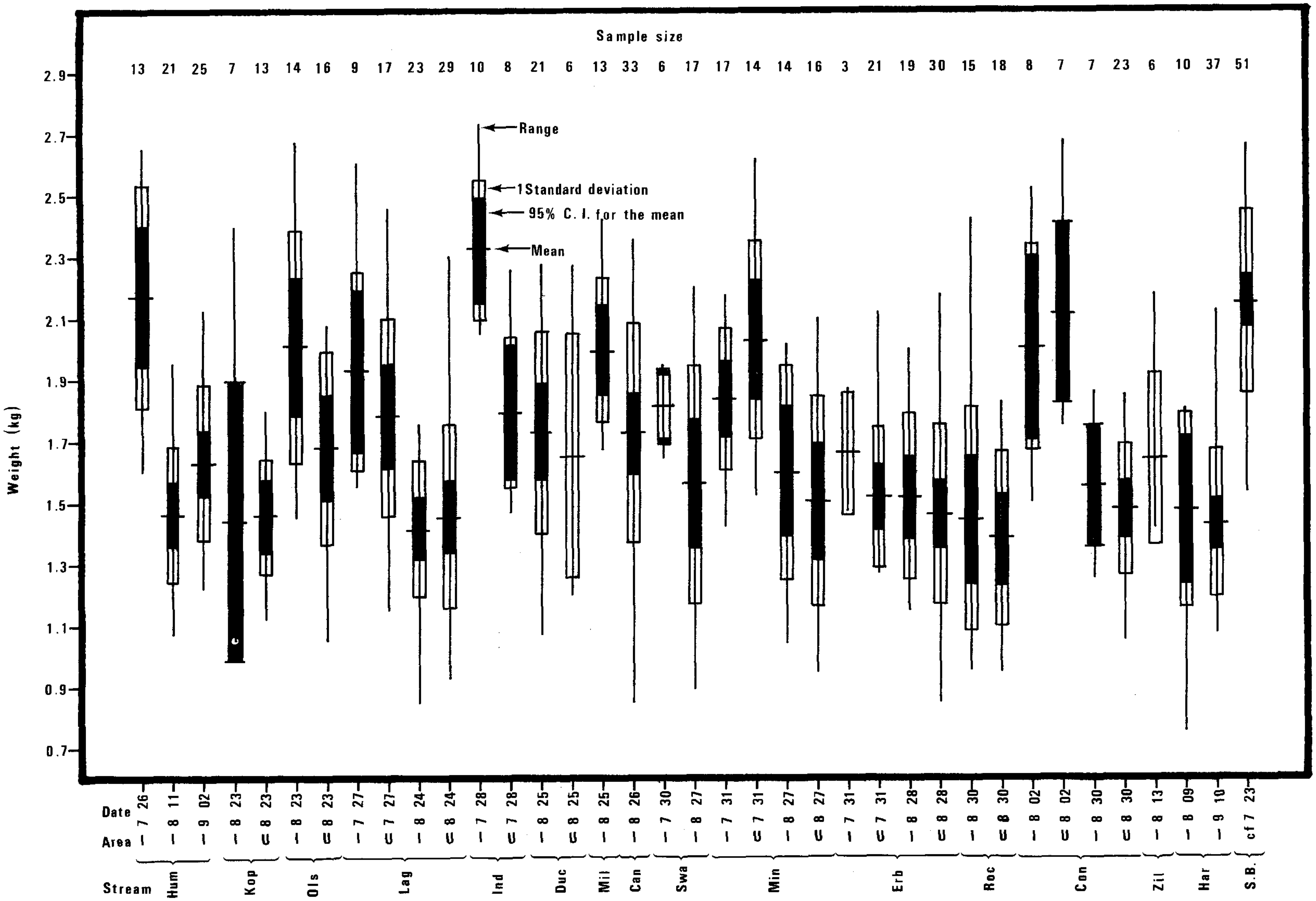

Fig. 8. Total live weight of Prince William Sound female pink salmon compared by stream, time and area (I = intertidal; U = upstream; c.f. = commercial fishery), 1976.

of comparison was of limited value. For example, comparison by analysis of variance of length and weight of the standard females to those of the July 26, 1976 Humpback Creek female sample show non-significant differences (i.e., for length, $F = 2.95$ (1, 62); $P = 0.09$ and for weight, $F = 0.06$ (1, 62); $P = 0.81$). Although no significant difference in length was observed between the female standard and the August 11, 1976 Humpback Creek female sample ($F = 3.28$ (1, 70); $P = 0.07$), a highly significant difference in weights for that date was obtained ($F = 90.85$ (1, 70); $P < 0.01$). These data imply that the same sub-population of fish were, by August 11, in the act of advanced spawning as reflected by their lower weight.

Use of the female standard data was of no value in other instances. For example, a comparison of lengths for early (July 26 and August 11) and late runs (September 2) to Humpback Creek yielded no significant difference. Early run length comparisons yielded $P = 0.09$ and 0.07, respectively, as provided in the previous paragraph, and late run length comparisons yielded $F = 3.04$ (1, 74); $P = 0.09$. Weights, however, were significantly lower than the female standard on August 11 ($P < 0.01$) and on September 2 ($F = 57.31$ (1, 74); $P < 0.01$). If stream-life factors were not known, these data could be erroneously interpreted as indicating one undifferentiated spawning population.

More meaningful results were obtained by comparing within-stream variation. For example, lengths of male and female Humpback Creek pink salmon (by sex) revealed no significant differences on the dates July 26 and August 11: ($F = 0.03$ (1, 44); $P = 0.86$ and $F = 0.06$ (1, 32); $P = 0.81$, respectively). However, significant decreases in weight for males ($F = 14.40$ (1, 44); $P < 0.01$) and for females ($F = 51.69$ (1, 32); $P < 0.01$) by August 11 indicated that the early run was nearly spawned-out.

By September 2, 1976 a significant increase in length was observed for females ($F = 8.97$ (1, 44); $P < 0.01$) and a non-significant increase in length was observed for males ($F = 4.08$ (1, 33); $P = 0.06$) when compared with the August 11 samples. During this same period (August 11 to September 2) a significant increase in female weight was observed ($F = 5.44$ (1, 44); $P = 0.02$), but male weight did not increase significantly ($F = 2.36$ (1, 33); $P = 0.14$). These observations implied that the late run had entered the stream. Figures 5, 6, 7, and 8 present the Humpback Creek data.

Similarly, early run males in Hartney Creek were significantly smaller that late run males ($F = 10.30$ (1, 42); $P < 0.01$), but early run females were not significantly smaller than late run females ($F = 3.98$ (1, 45); $P = 0.055$). Figures 5 and 6 show these data.

As shown in Figures 5 and 6 average length of fish was observed to increase with time in some streams (e.g., Humpback Creek), decrease in others (e.g., Koppen Creek) and remain essentially constant in still others (e.g., Lagoon Creek). In Koppen Creek, early and late run intertidal males could be separated on the basis of length ($F = 9.30$ (1, 56); $P < 0.01$), but other separations were not significant in systems exemplified. In Lagoon Creek where early and late runs of males and females could not be separated by length ($F = 0.71$ (3, 78); $P = 0.55$ and $F = 0.27$ (3, 74); $P = 0.85$, respectively), electrophoretic analyses revealed separations (Figure 9).

A four-way factorial analysis of variance (see Table 3) using the data summarized in Figures 5 and 6 yielded highly significant differences in length between streams ($F = 10.5$ (15, 1506); $P < 0.001$) and between the sexes ($F = 25.9$ (1, 1506); $P < 0.001$). There were no significant differences between intertidal and upstream spawners ($F = 0.013$ (1, 1506); $P = 0.91$), or between early and late spawners ($F = 3.0$ (1, 1506); $P = 0.08$).

The two-way interaction of stream and sex was not significant ($F = 1.08$ (15, 1506); $P = 0.37$), but a number of other two and three-way interactions were significant. For example, the stream-time interaction was highly significant ($F = 4.8$ (8, 1506); $P < 0.001$). Depending on the stream, either the early or the late run often consisted of larger fish. However, averaged over all streams, early and late fish did not differ in size. Similar interpretations can be applied to other significant two and three-way interactions. The four-way interaction was not significant ($F = 4.4$ (4, 1506); $P = 0.26$).

Electrophoretic analyses, however, exhibited some positive results. These data suggest that individual streams may be inhabited by sub-populations with little or no straying between them (Table 4 and Table 4a). In some streams there are genetic differences between early and late spawners as well as differences between intertidal and upstream spawners. Differences between early and late spawners are illustrated by the Humpback Creek intertidal spawners which were originally sampled on July 26, 1976 and again on September 2, 1976. The July samples contained the fast variant form of alpha-glycerophosphate dehydrogenase (AGP) at a frequency of .2435 (95% C.I. = $\pm$ .0972) while the September sample contained the same variant at a frequency of .0731 (95% C.I. = $\pm$ .0575). Even minor straying would tend to homogenize gene frequencies in a few generations, suggesting that there are two temporally isolated sub-populations in Humpback Creek. This suggestion is further supported by the relatively low Rogers' similarity of .978 between the two sub-populations (Table 5).

Differences between upstream and intertidal spawners are illustrated by July 27, 1976 upstream and August 24, 1976 intertidal runs to Lagoon Creek where AGP gene frequencies were .050 (95% C.I. = $\pm$ .049) and .213 (95% C.I. = $\pm$ .091), respectively. Figures 9 and 10 illustrate the differences

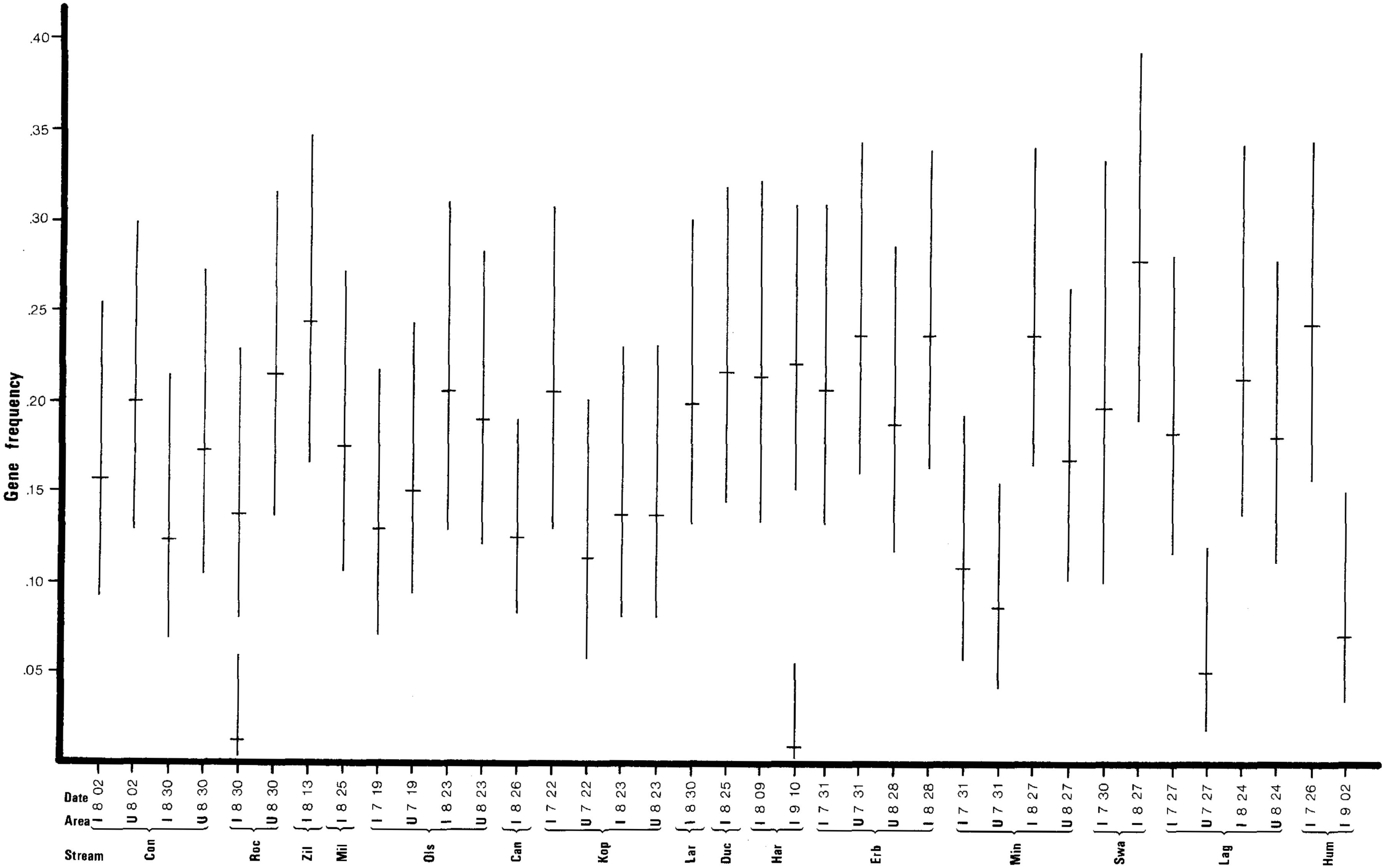

Fig. 9. Alpha-glycerophosphate dehydrogenase gene frequencies of Prince Willaim Sound pink salmon enclosed by 95 percent confidence intervals, compared by stream, time and area (I = intertidal; U = upstream). Rocky Creek (Roc) I 8-30 and Hartney Creek (Har) I 9-10 both contain two variant forms of the enzyme.

Table 3. Results of analysis of variance of mid-eye to fork length data of Prince William Sound pink salmon.

Source of Variation				Sum of Squares	DF	Mean Square	F	Signif. of F
Main Effects				223.902	18	12.439	10.622	< 0.001
Stream				185.270	15	12.351	10.547	< 0.001
Sex				30.280	1	30.280	25.857	< 0.001
Area				0.015	1	0.015	0.013	0.912
Time				3.490	1	3.490	2.981	0.088
2-Way Interactions				122.494	33	3.712	3.170	< 0.001
Stream	Sex			18.983	15	1.266	1.081	0.382
Stream	Area			20.579	7	2.940	2.511	0.016
Stream	Time			44.801	8	5.600	4.782	< 0.001
Sex	Area			3.690	1	3.690	3.151	0.080
Sex	Time			7.310	1	7.310	6.242	0.014
Area	Time			2.979	1	2.979	2.544	0.118
3-Way Interactions				35.203	21	1.676	1.431	0.093
Stream	Sex	Area		6.835	7	0.976	0.834	0.561
Stream	Sex	Time		10.503	8	1.313	1.121	0.360
Stream	Area	Time		13.011	5	2.602	2.222	0.049
Sex	Area	Time		0.621	1	0.621	0.531	0.478
4-Way Interactions				6.168	4	1.542	1.317	0.266
Stream	Sex	Area	Time	6.168	4	1.542	1.317	0.266
Explained				387.766	76	5.102	4.357	< 0.001
Residual				1763.601	1506	1.171		
Total				2151.367	1582	1.360		

Table 4. Measured gene frequencies and confidence intervals for alpha-glycerophosphate dehydrogenase, phosphoglucomutase, malate dehydrogenase A, malate dehydrogenase B, and aspartate aminotransferase.

Location	Date (1976)	Intertidal/ Upstream	N	AGP	2SE[1/]	PGM	2SE	MDHA	2SE	MDHB	2SE	AAT3	2SE
Constantine	8-30	I	40	.125	.074	.000		.000		.013	.025	.150	.080
Constantine	8-02	I	40	.158	.084	.000		.013	.025	.000		.143	.084
Constantine	8-02	U	40	.200	.089	.000		.000		.000		.153	.085
Constantine	8-30	U	40	.175	.0849	.000		.000		.000			
Rocky	8-30	I	40	.138	.077	.000		.025	.035	.013	.025	.244	.097
				.013	.025								
Rocky	8-30	U	40	.213	.091	.000		.025	.035	.000		.238	.096
								.013	.025	.013	.025		
Zillesenoff	8-13	I	40	.244	.097	.013	.025	.025	.035	.000		.098	.066
						.013	.025	.013	.025	.013	.025		
Millard	8-25	I	40	.175	.085	.000		.013	.025	.013	.025	.042	.058
										.025	.035		
Olsen	8-23	I	40	.205	.091	.000		.054	.053	.013	.025	.100	.067
										.013	.025		
Olsen	7-19	I	40	.129	.076	.000		.026	.036	.026	.036	.189	.091
Olsen	8-23	U	40	.188	.087	.013	.024			.025	.035	.200	.096
Olsen	7-19	U	40	.150	.080	.000		.000		.000		.175	.085
Cannery	8-26	I	76	.127	.054	.007	.013	.007	.013	.000		.129	.057
Koppen	7-22	I	40	.205	.091	.013	.025	.013	.025	.038	.042	.205	.091
								.025	.035				
Koppen	8-23	I	40	.138	.077	.000		.000		.013	.025	.175	.085
Koppen	7-22	U	40	.113	.071	.000		.013	.025	.025	.035	.163	.082
								.025	.035				

(Continued)

Table 4. Measured gene frequencies and confidence intervals for alpha-glycerophosphate dehydrogenase, phosphoglucomutase, malate dehydrogenase A, malate dehydrogenase B, and aspartate aminotransferase (continued).

Location	Date (1976)	Intertidal/ Upstream	N	AGP	2SE[1]	PGM	2SE	MDHA	2SE	MDHB	2SE	AAT3	2SE
Koppen	8-23	U	40	.138	.077	.000		.000		.025	.035	.213	.091
								.013	.025				
Larson	8-30	I	40	.200	.089	.000		.013	.025	.013	.025	.088	.097
Duck	8-25	I	39	.218	.093	.000		.090	.065	.000		.176	.088
Hartney	8-09	I	40	.213	.091	.000		.000		.000		.225	.093
								.013	.025				
Hartney	9-10	I	51	.221	.081	.000		.058	.046	.010	.019	.153	.073
				.010	.019								
Erb	7-31	I	40	.205	.091	.000		.000		.000		.118	.074
Erb	7-31	U	40	.238	.095	.000		.013	.025	.025	.035		
Erb	8-28	U	40	.188	.087			.013	.025	.013	.025	.250	.097
Erb	8-28	I	40	.238	.095	.025	.035	.025	.035	.000		.141	.079
Mink	8-27	I	40	.238	.095	.000		.063	.054	.025	.035	.167	.089
Mink	7-31	U	40	.088	.063	.000		.038	.042	.013	.025	.125	.078
Mink	7-31	I	42	.107	.067	.000		.000		.012	.024	.179	.084
Mink	8-27	U	39	.167	.084	.000		.013	.025	.000		.128	.076
Swanson	8-27	I	39	.278	.106	.013	.025						
Swanson	7-30	I	21	.190	.121	.000		.000		.048	.066	.194	.132
Lagoon	8-24	I	40	.213	.091	.000		.025	.035	.013	.025	.176	.088
Lagoon	7-27	I	39	.184	.090	.000		.000		.013	.025	.058	.065
Lagoon	8-24	U	40	.179	.087	.000		.025	.035	.000		.264	.104
Lagoon	7-27	U	40	.050	.049	.000		.000		.000			

(Continued)

Table 4. Measured gene frequencies and confidence intervals for alpha-glycerophosphate dehydrogenase, phosphoglucomutase, malate dehydrogenase A, malate dehydrogenase B, and aspartate aminotransferase (continued).

Location	Date (1976)	Intertidal/ Upstream	N	AGP	2SE 1/	PGM	2SE	MDHA	2SE	MDHB	2SE	AAT3	2SE
Humpback	9-02	I	41	.073	.058	.000		.037	.041	.012	.024	.159	.081
										.012	.024		
Humpback	7-26	I	40	.244	.097			.013	.025	.013	.025	.145	.081

1/ Plus or minus two standard errors encloses a 95 percent confidence interval (2SE). Groups with non-overlapping confidence intervals are genetically distinguishable from one another.

Table 4a. Measured gene frequencies and confidence intervals for lactate dehydrogenase 4, phosphoglucose isomerase 1, malic enzyme, and phosphoglucose isomerase 3.

Location	Date (1976)	Intertidal/ Upstream	N	LDH4	2SE[1]	PGI1	2SE	6PG	2SE	ME	2SE	PGI3	2SE
Constantine	8-30	I	40	.000		.000		.013	.025	.250	.097	.000	
Constantine	8-02	I	40	.000		.000		.075	.059			.000	
				.038	.042								
Constantine	8-02	U	40	.013	.025	.000		.050	.049			.013	.0248
Constantine	8-30	U	40	.025	.035	.000		.000		.162	.082	.000	
Rocky	8-30	I	40	.013	.024	.000		.038	.042	.191	.095	.000	
Rocky	8-30	U	40	.000		.050	.049	.038	.042	.250	.096	.000	
Zillesenoff	8-13	I	40	.000		.000		.024	.034	.256	.096	.000	
Millard	8-25	I	40	.013	.029	.000		.037	.042	.208	.166	.000	
Olsen	8-23	I	40	.000		.000		.000		.313	.104	.000	
Olsen	7-19	I	40	.013	.025	.026	.036	.026	.036			.000	
								.026	.036				
Olsen	8-23	U	40	.013	.025							.000	
Olsen	7-19	U	40	.000		.025	.035	.013	.025	.343	.117	.000	
Cannery	8-26	I	76	.021	.024	.000		.013	.019	.215	.072	.000	
Koppen	7-22	I	40	.025	.035	.013	.025	.000		.179	.087	.000	
				.013	.025			.154	.082				
Koppen	8-23	I	40	.025	.035			.000		.25	.097	.000	
Koppen	7-22	U	40	.013	.025	.000		.025	.035	.288	.101	.000	
Koppen	8-23	U	40	0.25	.035	.013	.025			.225	.093	.000	

(Continued)

Table 4a. Measured gene frequencies and confidence intervals for lactate dehydrogenase 4, phosphoglucose isomerase 1, malic enzyme, and phosphoglucose isomerase 3 (continued).

Location	Date (1976)	Intertidal/ Upstream	N	LDH4	2SE 1/	PGI1	2SE	6PG	2SE	ME	2SE	PGI3	2SE
Larsen	8-30	I	40	.000		.000		.063	.054	.152	.088	.000	
								.013	.025				
Duck	8-25	I	39	.000		.013	.025	.068	.058	.244	.097	.000	
Hartney	8-09	I	40	.013	.025	.000		.025	.035	.275	.099	.013	.025
Hartney	9-10	I	51	.000				.010	.019	.147	.070	.010	.019
Erb	7-31	I	40	.013	.025	.000		.026	.038	.300	.102	.000	
								.066	.057				
Erb	7-31	U	40	.000		.000		.025	.035	.263	.098	.000	
								.025	.035				
Erb	8-28	U	40	.000		.013	.025	.056	.054	.262	.099	.000	
Erb	8-28	I	40	.000		.025	.035	.000		.325	.105	.000	
Mink	8-27	I	40	.013	.025	.025	.035	.025	.035	.175	.085	.000	
										.025	.035		
Mink	7-31	U	40	.000		.025	.035	.100	.067	.275	.100	.000	
Mink	7-31	I	42	.000		.000		.024	.033	.225	.093	.000	
Mink	8-27	U	39	.000	.000	.000		.013	.025	.308	.104	.000	
Swanson	8-27	I	39	.013	.025	.013	.025	.013	.025	.205	.091	.000	
Swanson	7-30	I	21	.024	.047	.000		.048	.066	.238	.1314	.000	
								.167	.115				
Lagoon	8-24	I	40	.013	.025	.013	.025	.025	.035			.000	
Lagoon	7-27	I	39	.000		.000		.077	.060	.269	.100	.000	
								.077	.060				

(Continued)

Table 4a. Measured gene frequencies and confidence intervals for lactate dehydrogenase 4, phosphoglucose isomerase 1, malic enzyme, and phosphoglucose isomerase 3 (continued).

Location	Date (1976)	Intertidal/ Upstream	N	LDH4	2SE[1]	PGI1	2SE	6PG	2SE	ME	2SE	PGI3	2SE
Lagoon	8-24	U	40	.000		.013	.025			.167	.084	.000	
										.026	.036		
Lagoon	7-27	U	40	.000		.025	.035	.038	.042			.000	
Humpback	9-02	I	41	.000		.012	.024	.085	.062			.000	
Humpback	7-26	I	40	.013	.025			.038	.042	.131	.078		

1/ Plus or minus two standard errors encloses 95 percent confidence interval (2SE). Groups with non-overlapping confidence intervals are genetically distinguishable from one another.

Table 5. Genetic similarity matrix based on gene frequencies at 20 loci using Rogers' coefficient of genetic similarity among 37 populations of pink salmon 1/.

1 Hum I 9/2																																				
2 Swa I 7/30	77																																			
3 Swa I 8/27	77	85																																		
4 Min U 8/27	81	79	81																																	
5 Min I 7/31	86	85	82	82																																
6 Min U 7/31	93	71	70	80	80																															
7 Min I 8/28	77	79	92	73	77	69																														
8 Har I 9/10	79	74	89	76	76	68	90																													
9 Har I 8/09	73	85	86	83	81	70	77	76																												
10 Duc I 8/25	80	81	86	76	78	75	84	82	81																											
11 Lar I 8/30	79	78	83	78	76	72	77	84	72	77																										
12 Can I 8/26	81	81	85	87	90	78	76	77	77	73	79																									
13 Mil I 8/25	75	80	88	80	78	70	75	74	73	71	85	83																								
14 Zil I 8/13	73	75	90	82	75	73	80	79	80	80	78	78	79																							
16 Roc U 8/30	75	82	85	75	77	71	79	78	87	84	72	71	70	79																						
16 Roc I 8/30	81	83	85	75	85	70	78	76	82	74	76	82	78	70	83																					
17 Hum I 7/26	78	79	90	75	77	68	87	90	78	77	88	79	79	82	78	78																				
18 Lag U 7/27	89	81	78	86	92	88	77	78	82	77	83	88	84	76	82	86	82																			
19 Lag U 8/24	81	81	88	77	80	72	81	81	82	80	79	78	74	71	85	90	79	87																		
20 Lag I 7/27	78	80	80	83	77	80	68	69	77	77	85	77	86	81	72	70	73	84	73																	
21 Lag I 8/24	81	89	93	88	88	78	93	91	92	89	87	84	82	85	89	87	93	82	89	81																
22 Erb I 8/28	74	71	84	86	71	72	78	78	78	77	69	75	68	83	76	65	79	79	72	73	89															
23 Erb U 8/28	79	85	84	78	79	75	75	73	87	83	75	73	71	78	90	85	73	84	89	79	87	75														
24 Erb U 7/31	79	88	89	88	85	77	87	83	92	85	85	82	88	93	89	83	87	80	85	89	94	87	90													
25 Erb I 7/31	76	82	84	93	80	76	76	76	87	78	80	84	81	85	77	73	79	84	74	86	91	85	78	91												
26 Kop U 8/23	86	90	86	79	90	79	79	75	85	79	75	87	78	72	82	89	77	87	83	76	88	71	86	86	77											
27 Kop U 7/22	87	81	78	86	89	83	77	73	82	75	70	85	75	77	75	80	73	87	73	77	88	76	80	86	84	86										
28 Kop I 8/23	81	87	82	85	92	76	76	75	83	77	73	90	77	76	77	83	76	86	78	78	88	75	80	86	83	92	88									
29 Kop I 7/22	73	84	90	71	76	61	83	82	80	75	75	77	73	73	79	82	80	75	84	65	89	72	80	82	73	86	75	80								
30 Ols U 7/19	82	77	76	89	83	75	71	71	80	75	67	80	69	71	75	73	69	90	75	74	89	84	77	81	84	81	83	86	70							
31 Ols U 8/23	85	96	93	89	89	84	93	90	92	92	88	87	84	84	92	91	90	86	91	86	94	88	94	94	89	93	90	91	96	91						
32 Ols I 7/19	85	87	86	85	91	83	87	83	85	80	79	88	80	76	83	90	85	88	86	76	90	81	84	88	83	94	93	90	85	91	94					
33 Ols I 8/23	76	73	83	88	72	74	77	70	77	77	77	75	77	87	74	68	72	77	70	80	86	86	74	88	88	71	79	77	72	78	87	79				
34 Con U 8/30	78	86	85	84	85	69	81	86	84	75	89	90	89	78	78	88	87	84	93	82	90	75	81	81	84	88	78	89	88	79	95	88	78			
35 Con U 8/02	80	90	88	90	86	78	85	88	91	86	90	87	84	83	82	83	93	84	85	86	91	85	85	90	94	85	85	87	82	88	92	83	83	92		
36 Con I 8/02	84	84	81	91	85	84	78	81	81	82	88	88	83	78	76	83	87	83	84	86	84	80	81	85	87	87	85	86	77	86	88	83	79	89	90	
37 Con I 8/30	85	83	82	89	94	82	75	78	81	77	77	92	79	80	77	81	78	89	78	82	86	77	80	87	85	87	90	94	74	86	88	90	79	86	88	87
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36

1/ All of these values were between 0.900 and 1.000; therefore to increase the readability of this table only the last two digits are presented. Thus, 77 indicates a coefficient of 0.977.

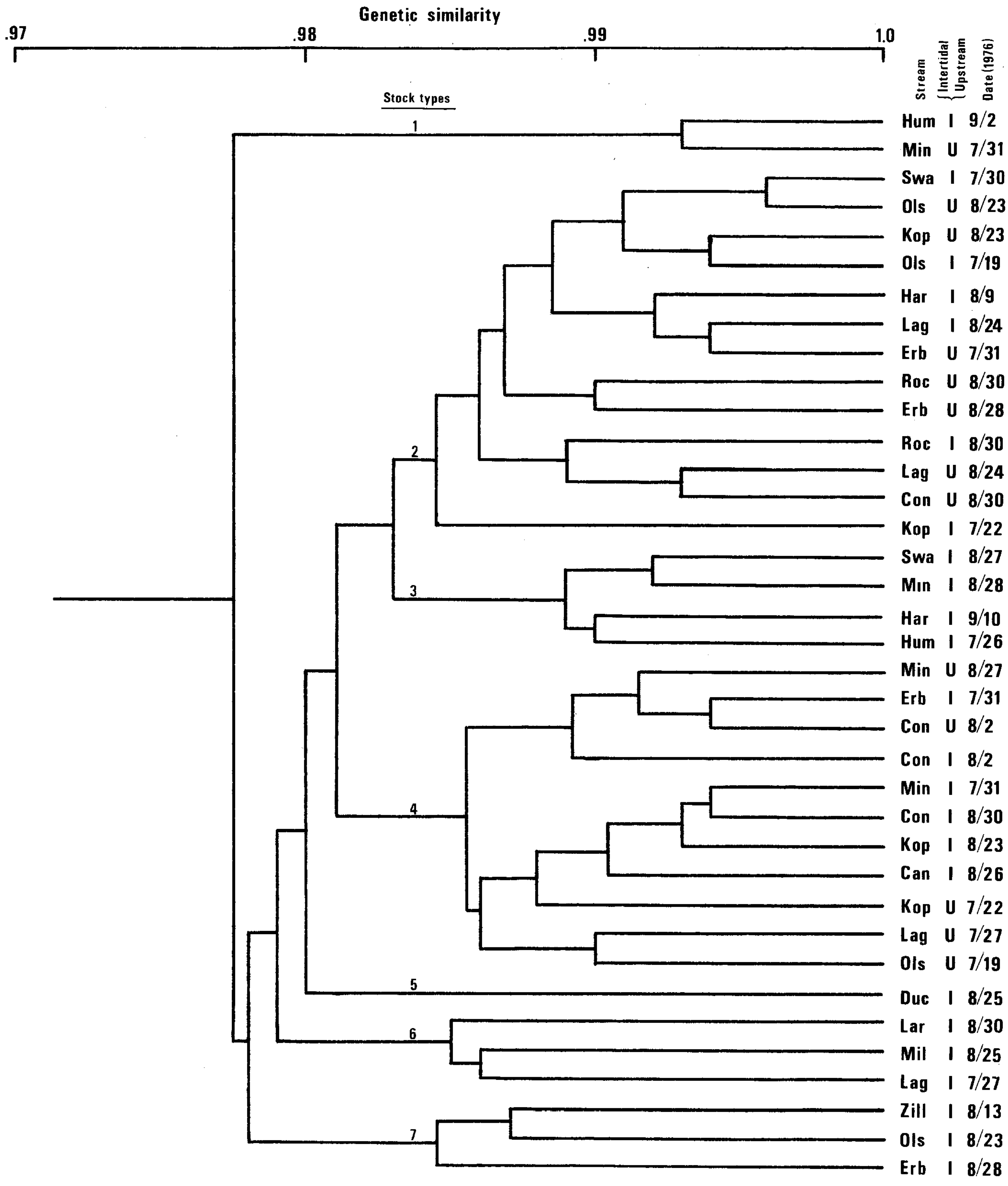

Fig. 10. Genetic similarity dendrogram of Prince William Sound pink salmon based on the unweighted average linkage method analysis of Rogers' genetic similarity coefficients.

between sub-populations and Table 6 presents a list of variant enzymes previously unseen in pink salmon. As expected, no significant gene frequency differences were found between sexes in the 37 sub-populations.

Electrophoretic analysis support within-stream variation of length-weight data when significant differences are observed. For example, the differences between the early and late runs to Humpback Creek are shown in Figure 11 by comparing length and alpha-glycerophosphate dehydrogenase (AGP) gene frequencies. Similarly, early and late runs of males to Hartney Creek differed significantly in length ($P < 0.01$) and the late run contained a variant form of AGP which was not observed in the early run (Figure 12).

Electrophoretic analysis support within group length comparisons to some extent. For example, in comparing grouped lengths by analysis of variance with the same groups found in Figure 10, lengths of Group 1 ("Hum I 9/2"; "Min U 7/31") males did not differ signficantly ($F = 0.02$ (1, 40); $P = 0.89$) but females did ($F = 5.78$ (1, 37); $P = 0.02$). Group 3 length did not differ significantly for males or females ($F = 2.49$ (3, 86); $P = 0.07$ and $F = 0.38$ (3, 77); $P = 0.77$, respectively). Group 6 males did not differ significantly in length ($F = 1.20$ (2, 75); $P = 0.31$), but females did ($F = 10.36$ (2, 39); $P < 0.01$). Conversely, Group 7 males differed significantly in length ($F = 5.31$ (2, 78); $P < 0.01$), whereas females did not ($F = 1.78$ (2, 36); $P = 0.18$).

Electrophoretic analysis support between-stream differences on paired comparisons, but not group comparisons. For example, the grand means and their 95% confidence intervals for male lengths in Groups 1, 3, and 6 (refer to Figure 10) were respectively: 461.83 ± 17.22; 461.54 ± 12.73, and 477.63 ± 15.74. No significant difference in length was found (e.g., comparing Groups 3 and 6, $t = 1.57$; $df = 1.66$, $P = 0.12$). The grand means and their 95% confidence intervals for female lengths in Groups 3 and 7 were, respectively: 475.79 ± 9.60 and 486.95 ± 12.18; these did not differ significantly ($t = 1.35$; $df = 118$, $P = 0.18$). Paired comparisons of lengths between Groups 1 and 3, however, revealed significant differences. When male and female lengths of "Hum I 9/2" and "Min I 8/27" (Figure 10) were compared (by sex), they differed significantly (i.e., for males $F = 6.20$ (1, 40); $P < 0.02$; for females $F = 9.87$ (1, 37); $P < 0.01$; only female length differed significantly when "Hum I 9/2" and "Har I 9/10" were compared ($F = 15.14$ (1, 60); $P < 0.01$), but male lengths did not ($F = 0.18$ (1, 28); $P = 0.67$). Again, comparison of lengths between "Hum I 9/2" and "Swa I 8/27" indicates that significant differences are observed between females ($F = 12.33$ (1, 40); $P < 0.01$), but not males ($F = 0.15$ (1, 37); $P = 0.70$).

Since spawning salmon were captured by round-hauling a visually-estimated sample (about 50), an unbiased sex ratio should have been obtained.

Table 6. Previously unseen protein variants in pink salmon observed from Prince William Sound, Alaska stocks. 1/

Enzyme	Relative Mobility
LDH4	125
AGP	50
MDH3	85
ME	115
PGM	70
PGI3	90

1/ The common mobility is defined as 100; therefore, a relative mobility of 125 indicates that the variant protein migrates 25 percent faster than the common type and vice versa.

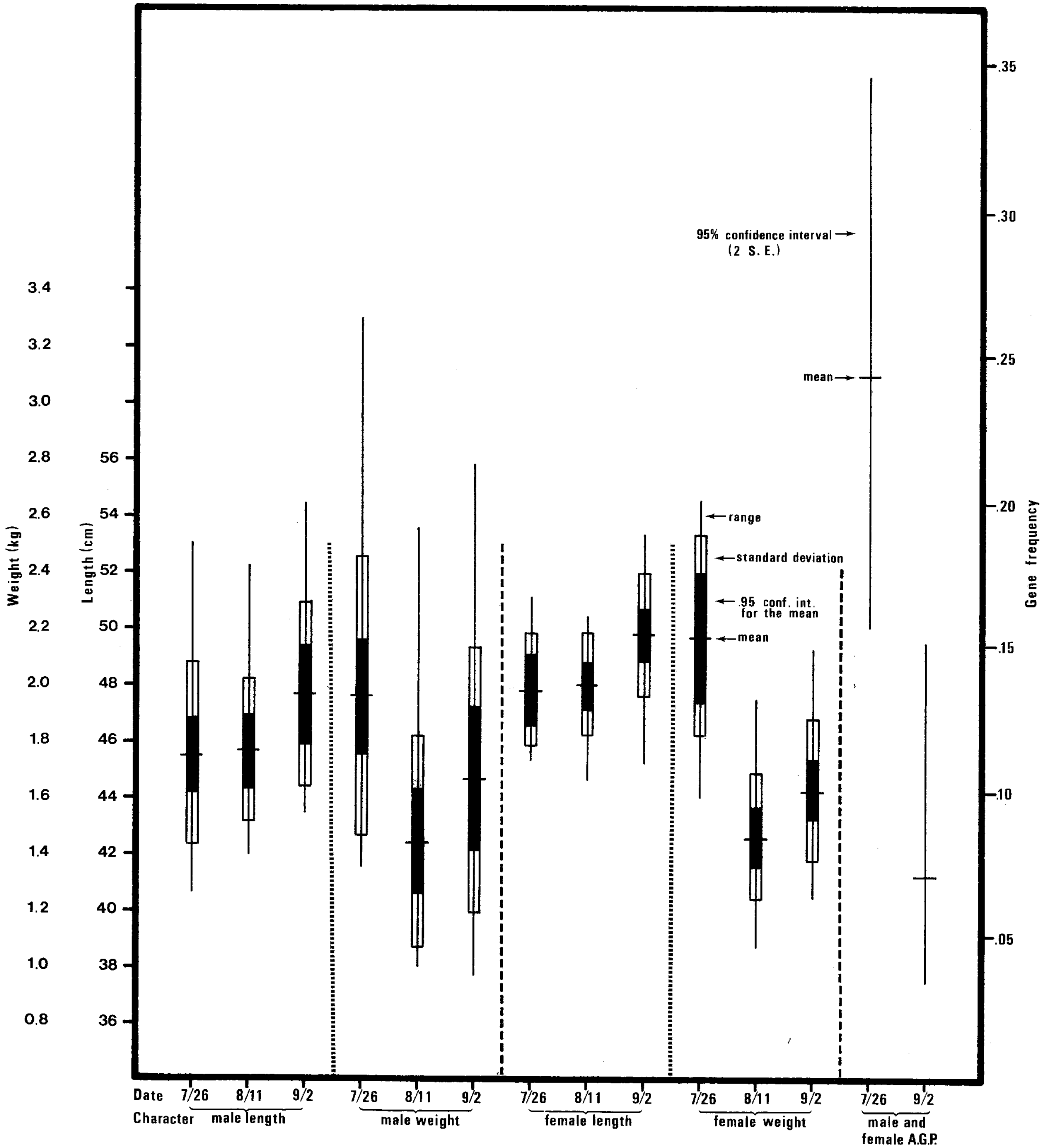

Fig. 11. Mid-eye to fork lengths and total live weights of Humpback Creek pink salmon compared to gene frequencies of alpha-glycerophosphate dehydrogenase (AGP) by time, 1976.

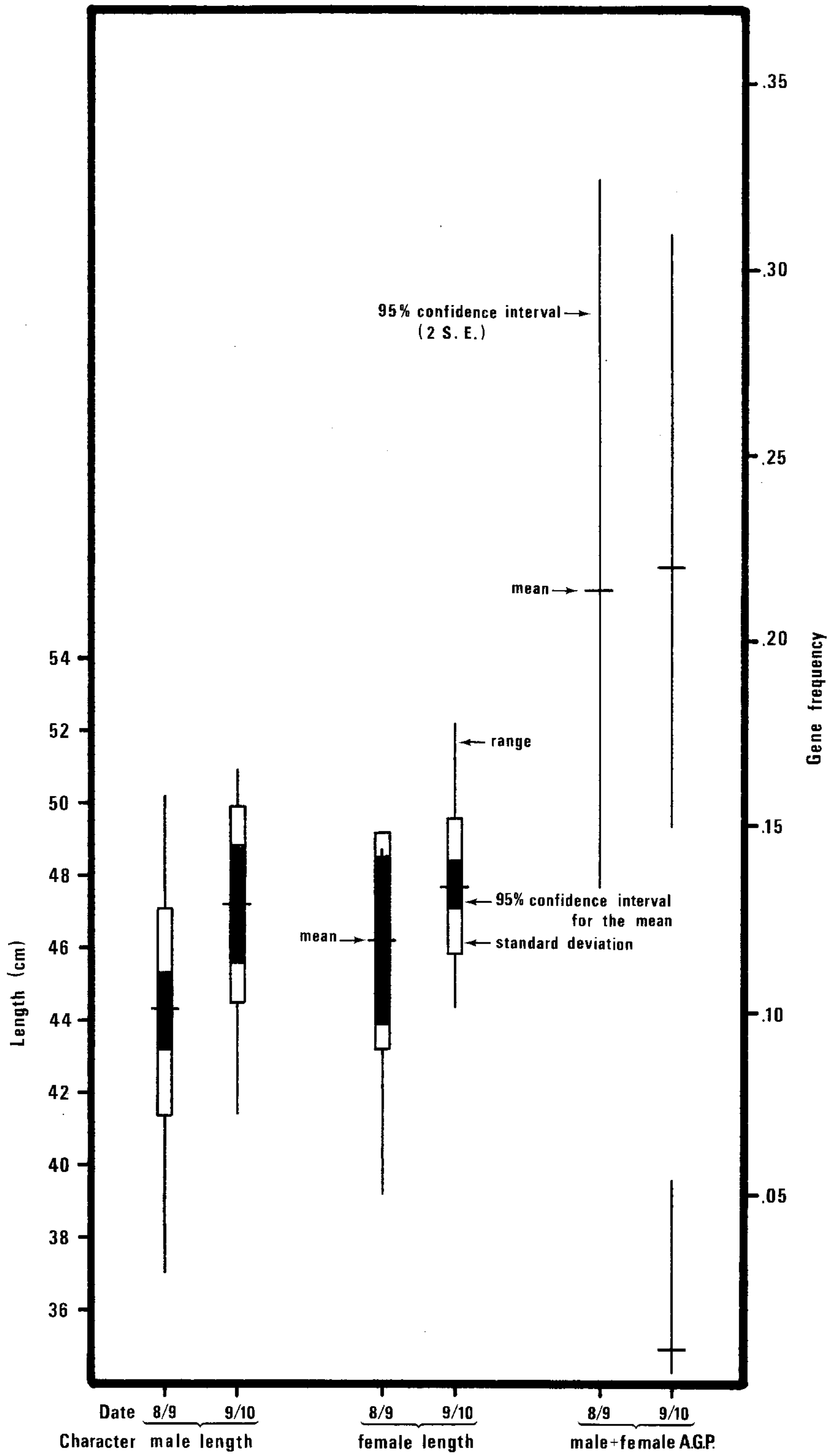

Fig. 12. Mid-eye to fork lengths of Hartney Creek pink salmon compared to gene frequencies of alpha-glycerophosphate dehydrogenase (AGP) by time, 1976.

Chi-square analysis of data indicated that males significantly outnumbered females, about 1.77 to 1, except during the late upstream run when no significant difference was exhibited. Table 7 presents the findings.

DISCUSSION

The use of length/weight data for identifying sub-populations within and between systems has limited applicability. While clear separations can be made in certain instances, (e.g., Humpback Cr.), real genetic variation can also be obscured (e.g., Lagoon Cr.). The interesting result of this type of analysis is that real differences in size-stream-timing-area relationships do exist (within stated probability levels) in the relatively small geographic area of Prince William Sound. When highly significant length data are compared with electrophoretic analysis, the two forms of investigation are shown to be mutually supportive as shown in Figures 11 and 12. Certain enzymes, by virtue of their polymorphism, are more useful than others in disclosing genetic differences. These differences are displayed as biochemical variation upon staining gels following electrophoresis. Alpha-glycerophosphate dehydrogenase (AGP) is highly polymorphic and reliably resolvable, hence, it is a useful locus in characterizing populations. As exhibited in Figure 9, there is no overlap between several of the confidence intervals, indicating significant differences at the 95% level. Not only are there differences between sample stream sites, but for some sites the AGP frequencies varied with time suggesting temporal separation of sub-populations from the same site. This evidence supports the contention that not all of the streams contain single panmictic units, but some are composed of genetically identifiable sub-populations that may vary both spatially and temporally.

Low frequency protein variants are also useful in characterizing populations when significant differences are observed. For example, both Lagoon Creek intertidal samples showed MDH-B variation (although not significant) while the upstream (freshwater) samples were monomorphic for the common protein type. Both Erb Creek upstream samples showed MDH-B variation, while neither intertidal sample showed any. The same held true for Rocky Creek upstream and intertidal in which the two sites showed differing (although not significant) rare variants for AGP, MDH-A, and MDH-B. These results support the hypothesis that many of the streams may have spatially isolated sub-populations. The results also indicate that larger sample sizes are necessary (i.e., n = 100 rather than 40).

If each stream actually contained a freely interbreeding panmictic unit, the similarity indices (Table 5) between samples from the same streams should all be relatively high, while the indices between samples from different streams should be lower. This would lead to a clustering effect in which

Table 7. Chi-square analyses of partitioned-run sex ratios of spawning pink salmon captured from 16 streams in Prince William Sound, Alaska during 1976.

Test[1]		n Males	n Females	N	X^2	P
I.	Early intertidal	278	102	380	80.59	< 0.001
II.	Early upstream	185	96	281	27.56	< 0.001
III.	Late intertidal	322	245	567	10.19	< 0.001
IV.	Late upstream	134	145	279	0.36	0.549

V

	♂	♀	Total
Early intertidal	278	102	308
Early upstream	185	96	281
Total	463	198	661

$X^2 = 3.79;\ P = 0.052$

VI

	♂	♀	Total
Late intertidal	322	245	567
Late upstream	134	145	279
Total	456	390	846

$X^2 = 5.43;\ P = 0.020$

Table 7. Chi-square analyses of partitioned-run sex ratios of spawning pink salmon captured from 16 streams in Prince William Sound, Alaska during 1976 (continued).

		♂	♀	Total
VII	Early intertidal and upstream	463	198	661
	Late intertidal and upstream	456	390	846
	Total	919	588	1,507

$X^2 = 39.97$; $P < 0.001$

1/ Tests I to IV are single classification; V to VII are two-way classification for independence. Yates' correction for continuity is applied to all tests.

all samples from the same stream would be on the same branch of the dendrogram (Figure 10) at a high similarity value. This is not the case, however. No obvious clustering is observable, a result directly predictable from the diverse intrastream gene frequencies.

The relationships of the sub-populations in Figure 10 reflect all the accumulated frequency differences of all the protein variants. These relationships may be patterned by factors such as geographic isolation, historic patterns of glaciation, and random drift. Whatever the cause, the similarities among natural populations outlined in Table 5 and Figure 10, may be used as parameters in managing the above-listed stocks.

The unequal sex ratio displaying numerical male dominance for three of the four partitioned run segments, appears to be a population density and/or behavioral attribute rather than a sampling artifact. Kirkwood (1962) describes spawning behavior of pink and chum salmon at Olsen Creek, Prince William Sound, Alaska in which two or more males, one dominant, attend a ripe female. Helle et al. (1964) indicates that stream-life of pink salmon at Olsen Creek, Prince William Sound, Alaska, ranged from 21 days during the early run to 5 days in the late run. The significant difference in sex ratio between early and late runs quantified in Chi-square comparisons (Table 7) may be related to the observations of Kirkwood and Helle et al.

I do not know whether the sex ratios observed in partitioned run segments are valid or whether a more balanced sex ratio was masked by stream-life factors. For example, spent or partially spent males, perhaps dominant during early and late intertidal and early upstream runs, may subsequently assume a subordinate role in spawning activities.

I feel that the information and knowledge gained in this study may prove useful in maintaining genetic integrity of hatchery donor stocks and isolating other populations as wild stock preserves.

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